

In the Claims:

Please cancel claims 1-18.

Please add the following new claims:

--19. A method of determining the proportion of apoptotic cells in a culture, comprising:

(A) transiently transfecting a population of mammalian cells with a plasmid containing a sequence of interest;

(B) co-transfecting the population of cells with a plasmid containing DNA coding for a fluorescent marker protein;

(C) culturing the cells in a suitable nutrient medium so that the DNA sequence of interest or its expressed polypeptide exerts its potential activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that any fluorescent protein expressed remains in the cells, while the apoptotic DNA fragments diffuse out;

(E) measuring the proportion of apoptotic cells by measuring total DNA content remaining subsequent to (D);

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(F) also, measuring the proportion of transfected cells by quantitating the fluorescent marker protein contained in the cells harvested in (D); and

(G) comparing the values obtained in (E) and (F);

thereby, determining the proportion of apoptotic cells in the transfected population.

20. The method of claim 19, wherein the transient transfection method used in (A) and (B) is a receptor-mediated endocytosis method using polyethyleneimine and inactivated Adenovirus.

21. The method of claim 19, wherein the fluorescent marker protein in (B) is Green Fluorescent Protein.

22. The method of claim 19, wherein the proportion of apoptotic cells are measured in (E) with a DNA binding stain.

23. The method of claim 22, wherein the DNA binding stain is propidium iodide.

24. The method of claim 19, wherein the fixing and permeabilization in (D) is achieved with paraformaldehyde and ethanol, respectively.

25. The method of claim 19, wherein the measuring in (E) and (F) is achieved by fluorescence activated throughflow cytometry analysis.
26. A method of determining whether a gene of interest has an effect on apoptosis of cells in a culture, comprising:
- (A) transiently transfecting a population of mammalian cells with a plasmid containing a sequence of interest, thereby obtaining population X; and transiently transfecting another population of the same cells with a control plasmid, thereby obtaining a population Y;
 - (B) co-transfecting the population of cells with a plasmid containing DNA coding for a fluorescent marker protein;
 - (C) culturing the cells in a suitable nutrient medium so that the DNA sequence of interest or its expressed polypeptide exerts its potential activity on the apoptosis of the cell;
 - (D) harvesting the cells from (C) and fixing and permeabilizing the cells so that any fluorescent protein expressed remains in the cells, while the apoptotic DNA fragments diffuse out;

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(E) measuring the proportion of apoptotic cells by measuring total DNA content remaining subsequent to (D);

(F) also, measuring the proportion of transfected cells by quantitating the fluorescent marker protein contained in the cells harvested in (D);

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells; and

(H) comparing the calculated proportion of apoptotic cells obtained in (G) for the populations X and Y.

thereby, determining whether the gene of interest affects the proportion of apoptotic cells in the transfected population.

27. The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative signal transmission molecule of a receptor for a survival factor particular to a tumor cell.
28. The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative receptor for a survival factor particular to a tumor cell.

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29. The method of claim 28, wherein the dominant negative receptor is the IGF-1 receptor.

30. The method of claim 28, wherein the dominant negative receptor is the FGF receptor.

31. The method of claim 28, wherein the dominant negative receptor is the PDGF receptor.

32. A method of determining the effect of a test substance on the pro- or anti-apoptotic activity of a gene of interest, comprising:

(A) transiently transfecting two populations of mammalian cells with an identical plasmid containing a sequence of interest;

(B) co-transfecting the population of cells with a plasmid containing DNA coding for a fluorescent marker protein;

(C) culturing one population of transfected cells in a suitable nutrient media containing a test substance, thereby obtaining a population X; and incubating the other population of transfected cells in a suitable medium lacking the test substance, thereby obtaining a population Y;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that any fluorescent protein expressed remains in the cells, while the apoptotic DNA fragments diffuse out;

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(E) measuring the proportion of apoptotic cells by measuring total DNA content remaining subsequent to (D);

(F) also, measuring the proportion of transfected cells by quantitating the fluorescent marker protein contained in the cells harvested in (D) ;

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells;

(H) comparing the calculated proportion of apoptotic cells obtained in (G) for the populations X and Y; and

(I) selecting those test substances which exhibit a synergistic activity;

thereby, determining whether the test substance has an effect on the proportion of apoptotic cells transfected with the gene of interest.

33. The method of claim 32, wherein the mammalian cells in (A) are tumor cells; the gene of interest in (A) is a dominant negative version of a signal transmission molecule of a receptor for a survival factor particular to a tumor cell, which causes the inhibition or absence of a survival factor function; and the test substance exhibits activity synergistic with the inhibition or absence of the survival factor function.

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34. The method of claim 32, wherein the mammalian cells in (A) are tumor cells; the gene of interest in (A) is a dominant negative version of a receptor for a survival factor particular to a tumor cell, which causes the inhibition or absence of a survival factor function; and the test substance exhibits activity synergistic with the inhibition or absence of the survival factor function.

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35. The method of claim 34, wherein the receptor for a survival factor is the IGF-1 receptor.

36. The method of claim 34, wherein the receptor for a survival factor is the FGF receptor.

37. The method of claim 34, wherein the receptor for a survival factor is the PDGF receptor.

38. The method of claim 32, wherein the test substance in (C) acts synergistically with chemotherapy.

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39. The method of claim 33, wherein the test substance in (C) acts synergistically with chemotherapy.

40. The method of claim 34, wherein the test substance in (C) acts synergistically with chemotherapy.

41. A kit for determining the proportion of apoptotic cells in a culture which comprises a carrier means being compartmentalized to receive in close confinement one or more container means wherein:

(A) the first container holds one or more components sufficient for transfection;

(B) another container holds a plasmid containing the sequence coding for the fluorescent marker protein;

(C) another container holds an empty vector for inserting the DNA sequence of interest and for control measurements;

(D) another container holds the primary fixing solution;

(E) another container holds the secondary fixing/permeabilizing solution;

(F) another container holds washing solution(s); and

(G) a final container holds a DNA-binding stain.

42. The kit in claim 41, wherein the transfection components in (A) are sufficient to achieve receptor-mediated endocytosis using polyethyleneimine and psoralen/UV-inactivated Adenovirus.

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43. The kit in claim 41, wherein the fluorescent marker protein in (B) is Green Fluorescent Protein.
44. The kit in claim 41, wherein the primary fixing solution in step (D) is 2% paraformaldehyde and the secondary fixing/permeabilization solution in (E) is 70% ethanol.
45. A method of expression cloning of genes which modulate apoptosis comprising:
- (A) transiently transfecting a complete cDNA expression library into cells;
 - (B) co-transfecting the population of cells with a plasmid containing DNA coding for a fluorescent marker protein;
 - (C) culturing the cells in a suitable nutrient medium so that the DNA sequence of interest or its expressed polypeptide exerts its potential activity on the apoptosis of the cell;
 - (D) harvesting the cells from (C) and fixing and permeabilizing the cells so that any fluorescent protein expressed remains in the cells, while the apoptotic DNA fragments diffuse out;
 - (E) measuring the proportion of apoptotic cells by measuring total DNA content remaining subsequent to (D);

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